SDS-PAGE for protein electrophoresis

Aim

Separation of proteins according to size by electrophoresis using a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins.

Procedure

Sample Preparation

1. Prepare suitable amount of sample buffer (25 μl per reaction).
2. Determine protein concentration with NanoDrop.
3. Calculate required dilutions needed to get 150 μg protein in 75 μl of solution.
4. In the fume hood, add 25 μl of the sample buffer (blue) to 75 μl of protein samples (total volume of 100 μl). Centrifuge samples at 13000 rpm for 60 seconds.
5. Incubate samples for 5 minutes in a dry heating block at 90 °C.
6. Centrifuge samples at 13000 rpm for 60 sec.
7. Store all samples at −20 °C.

Table 1: Materials for 5x Sample Buffer

10 % w/V	SDS
10 mM	Dithiothreaitol, or beta-mercapto-ethanol
20 % v/v	Glycerol
0.2 M	Tris-HCl, pH 6.8
0.05 % w/v	Bromphenolblue

Gel preparation

Separating gel

1. Prepare the gel casts in holders. Fill the holder with water to check for leakage after 5 min. After leakage check, pour all water from gel casts and dry residual with paper towel.
2. To prepare separation-gel, ix the reagents listed in the table below with the proper volumes to receive a gel with desired composition.
3. NOTE! Add TEMED and APS last, solidification of the gel will occur when these reagents are added.
4. Pipette gel mixture into gel casts up to a height approximately 1 cm below the gels comb.
5. Fill the remaining cast with isopropanol (if not available use distilled water).
6. Allow to harden for 30-60 min.
7. Pour all isopropanol (or water) from gel casts and dry residual with paper towel.

- Ammonium Persulfate (APS) - 20 mg/ml
- Acrylamide 30 % solution
- TEMED
- **Separating Gel Buffer** - 1.5 M Tris-HCl pH 8.8 with 0.4% SDS. Stored at 4 °C.
- **Stacking Gel Buffer** - 0.5 M Tris-HCl pH 6.8 with 0.4% SDS. Stored at 4 °C

Table 2: Percentage of Acrylamide depending on protein size.

Acrylamide [%]	M.W. Range [kDa]
7	50 - 500
10	20 - 300
12	10 - 200
15	3 - 100

Table 3: Separating gel composition.

Acrylamid [%]	6 % [ml]	8 % [ml]	10 % [ml]	12 % [ml]	15 % [ml]
H_20	5.2	4.6	3.8	3.2	2.2
Acrylamide/Bis-acrylamide (30 % / 0.8 % w/v)	2	2.6	3.4	4	5
1.5 M Tris, pH 8.8	2.6	2.6	2.6	2.6	2.6
10 % (w/v) SDS	0.1	0.1	0.1	0.1	0.1
10 % (w/v) ammonium persulfate (AP)	0.1	0.1	0.1	0.1	0.1
TEMED	0.01	0.01	0.01	0.01	0.01

Stacking gel

1. To prepare stacking-gel, in a tube mix the reagents listed in table 4 below with the proper volumes to receive a gel with desired composition.
2. NOTE! Add TEMED and APS last, solidification of the gel will occur when these reagents are added.
3. Pipette gel mixture on top of separating gel to fill cast and remove all air bubbles.
4. Fill the remaining cast with isopropanol (if not available use distilled water).
5. Insert the comb whilst the gel is still in liquid form. The comb will form wells to load samples when the gel turns solid.
6. Allow to harden for 45-60 min.

Electrophoresis

This section is taked and modified from the protocol provided by Kristian Dreij, Hanna Karlsson in the course "Applications of methods in toxicology" at Karolinska Institute.

Table 4: Stacking gel composition

Material	Amount [ml]
H_20	2.975
0.5 M Tris-HCl, pH 6.8	1.25
10 % (w/v) SDS	0.05
Acrylamide/Bis-acrylamide (30 % / 0.2 % w/v)	0.67
10 % (w/v) ammonium persulfate (AP)	0.05
TEMED	0.005

Running the gel

1. Mount the gels into the tank, remove combs and fill the inner chamber of the tank with 1x running buffer to the top. Fill a third of the volume in the outer chamber of the tank with 1x running buffer
2. Pipette 2 μl ladder and 10 μl sample into each well.
3. Connect power pack (red-red, black-black) and run at 100 V, 60 min or until the blue line almost reaches the bottom of the gel.
4. Once electrophoresis has finished, remove gels from the glass plates.
5. Carefully wash all equipment used for electrophoresis with water.

10x Running buffer

Dissolve 30.0 g of Tris base, 144.0 g of glycine, and 10.0 g of SDS in 1000 ml of H_2O. The pH of the buffer should be 8.3 and no pH adjustment is required. Store the running buffer at room temperature and dilute to 1X before use.

Coomassie Blue Staining

1. Prepare the staining solution containing 0.1 % Coomassie Blue in 40 % ethanol, 10 % acetic acid.
2. After electrophoresis, incubate 1 or 2 gels in a staining container containing 100 ml Coomassie Blue staining solution.
3. Incubate at room temperature for 1 hour until bands are visible.
4. Decant the stain and rinse the gel once with deionized water.
5. Prepare 100 ml destaining solution containing 10 % ethanol and 7.5 % acetic acid.
6. Gently shake the gel at room temperature on an orbital shaker until the desired background is achieved.

Sources

SDS-PAGE protocol by Assay-protocol.com.
The protocol written by Kristian Dreij, Hanna Karlsson in the course "Applications of Methods in Toxicology" at Karolinska Institute.